are repeatedly used (*6*). The minimum 140-JND criterion was also applied to a new probe item to ensure that it differed substantially in frequency from list items.

10. After the experiment was completed, we discovered that, for five-sound lists, serial position and the delay value preceding the probe had been inadvertently confounded: because of a programming error, the 4-second delay occurred much more frequently at serial positions 1 and 2 than at 3, 4, and 5; the opposite was true for the 1-second delay. This confounding resulted in a spuriously highly significant delay effect ($\chi^2 = 18.4$, $P < .0001$) and may have also been responsible for a significant bias toward responding "old" ($\chi^2 = 8.9$, $P < .005$). For all other list lengths, the two delay values occurred equally often at each serial position, and the differences in their effects were small and nonsignificant ($P > .05$). Also, no significant response bias occurred in any of these other list lengths. The five-sound-list data for serial positions 1 through 5–96.6, 92.2, 82.4, 68.5, and 61.1 percent correct responses, respectively–nevertheless showed the same general trends seen in Fig. 1A, though their absolute values were elevated because of the bias toward responding "old." When the data were corrected for bias [according to techniques described by D. M. Green and J. A. Swets, *Signal Detection Theory and Psychophysics* (Wiley, New York, 1966)], the adjusted percentages of correct responses across the five serial positions—95.4, 91.1, 79.1, 62.0, and 53.3 percent—fell very close to the plotted values for other data in Fig. 1A.
11. Absolute values for human data may vary considerably across studies depending on the type of material to be remembered, though the recency effect will continue to be shown. Recognition probabilities for lists of single letters (*4*) substantially exceeded the values listed here, but recognition probability for the first continuous frequency signal in a list of similar signals, as studied by D. W. Massaro [*J. Exp. Psychol.* **83**, 238 (1970)], fell well below the listed values.
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15. If our experiment is conceptualized in terms of signal detection concepts (*14*), and a strength (item familiarity) theory is used for recognition memory (*3*), old and new probe items form independent but partially overlapping normal distributions (with equal variances and with a difference of d′ in the means) along a decision axis in which old (previously heard) probes have greater mean strength (familiarity) than new probes. With equal probability of old and new probe items and a symmetrical payoff matrix, the maximum likelihood criterion would be located at the intersection of the ordinates of the two distributions. If this criterion is then used in recognition decisions, unbiased responding will result, as indeed happened.
16. See C. A. Boneau and J. L. Cole [*Psychol. Rev.* **74**, 123 (1967)] for a discussion of the use of the maximum likelihood criterion when trial-by-trial information on the accuracy of responses is available, as is the case in most animal studies including the present one; and E. A. C. Thomas and D. Legge [*ibid.* **27**, 65 (1970)] on the use of a probability matching criterion (matching the ratio of the responses in each category to the a priori ratio of occurrence of the categories) when trial-by-trial information is not available, as is the case in many human studies.
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* Reprint requests should be sent to L.M.H.

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Dopamine-Sensitive Adenylate Cyclase: Location in Substantia Nigra

Abstract. *A dopamine-sensitive adenylate cyclase with characteristics similar to those measured in the striatum is present in the rat substantia nigra. Destruction of dopamine cell bodies by intranigral 6-hydroxydopamine application failed to abolish the response of nigral adenylate cyclase to dopamine. In contrast, brain hemitransection between the striatum and substantia nigra, or a more circumscribed lesion of striatonigral pathways, abolished the dopamine stimulation of adenylate cyclase in the substantia nigra. These results suggest that dopamine receptors within the substantia nigra are not located on dopamine cell bodies but are associated with a pathway, containing γ-aminobutyric acid or substance P, which projects from forebrain structures to the substantia nigra.*

The substantia nigra (SN) is a strategic relay center for the modulation of dopamine (DA) function in the central nervous system (*1*). Dopamine-containing efferent projections from the SN have been extensively characterized (*2*). In addition, neurons containing γ-aminobutyric acid (GABA) (*3*), substance P (*4*), acetylcholine (*5, 6*), and serotonin (*7*) have been found in the SN, but relatively little is known about the synaptic interactions of these neurons.

In an effort to unravel possible sites of interneuronal regulation in the SN, we pursued the idea that DA, released from nigral dendrites (*8*), may interact with receptors in the immediate vicinity. Since DA receptors are apparently associated with a DA-sensitive adenylate cyclase (*9*), and since the presence of such an adenylate cyclase has been demonstrated in the SN (*10*), it is likely that DA serves a neuromodulatory function in this region. The suggestion has been made that in the SN DA may influence the activity of the very same neurons from which it is released by interacting with dendritic or somatic DA receptors, which have been termed autoreceptors (*11*). Alternatively, it is likely that nondopaminergic neuronal components in the SN may contain receptors for this transmitter.

On the basis of measurements of DA-sensitive adenylate cyclase activity, we now provide evidence that most, if not all, nigral DA receptors are present in cells other than DA neurons. In the first series of experiments, rats received unilateral nigral injections (Fig. 1) of 6-hydroxydopamine (6-OHDA) and were killed 10 to 15 days later to study the adenylate cyclase activity of the SN. To ascertain the extent of DA neuron destruction caused by 6-OHDA, we assayed tyrosine hydroxylase (TH) activity in the striatum, a terminal projection area of nigral DA neurons.

In homogenates of untreated SN, the threshold concentration of DA for stimulation of adenosine 3′,5′-monophosphate (cyclic AMP) synthesis was $10^{-6}M$ (Fig. 1); with $5 \times 10^{-6}M$ DA, stimulation was virtually maximal. Norepinephrine (NE) was approximately ten times less potent than DA; threshold stimulation was obtained at $10^{-5}M$ NE.

In homogenates of 6-OHDA–treated SN, the absolute and relative potencies of both DA and NE did not significantly differ from values obtained in untreated controls (Fig. 1). Since the 6-OHDA treatment resulted in an 80 to 90 percent loss of striatal TH (Fig. 1), the data demonstrate that in the SN the presence of DA-sensitive adenylate cyclase is independent of the amount of DA cell bodies.

In a second series of experiments a complete cerebral hemitransection at the diencephalic-mesencephalic junction was performed in rats 7 days before they were killed (*12*). In the SN of the lesioned side, the basal activity of adenylate cyclase (30 ± 2.0 pmole mg^{-1} min^{-1}) was comparable to that of the intact side (28 ± 1.5 pmole mg^{-1} min^{-1}). In homogenates of the SN from the intact side, DA ($10^{-5}M$) produced a nearly twofold increase in cyclic AMP synthesis (54 ± 4.1 pmole mg^{-1} min^{-1}). In contrast, DA failed to stimulate the adenylate cyclase activity of SN from the lesioned side (38 ± 4.2 pmole mg^{-1} min^{-1}). When the hemitransection was done posterior to the SN (*13*), DA stimulation of adenylate cyclase was not significantly altered.

These results provide biochemical evidence that DA-dependent adenylate cyclase is located in neurons connecting the forebrain with the SN. Since our data tend to exclude the possibility that DA-dependent adenylate cyclase is directly associated with DA cell bodies or dendrites (*14*), a third series of experiments was performed to determine more specifically what population of SN neurons contains the major proportion of DA-sensitive adenylate cyclase. One of the most significant biochemical changes reported in the SN after hemitransection is the loss of GABA and its synthesizing en-

zyme glutamic acid decarboxylase (*3*); therefore we attempted to more selectively eliminate the GABA input to the SN.

We developed a technique of producing small unilateral electrolytic lesions anterior and lateral to the SN which resulted in a 70 percent loss of GABA in the ipsilateral SN without causing any significant change in nigrostriatal TH (see Table 1). We also measured the substance P content in the SN after these lesions, since it was recently demonstrated that the substance P content of the SN is significantly reduced after hemitransections between SN and striatum (*15*). As shown in Table 1, our electrolytic lesions of striatonigral projections caused the nigral substance P content to decrease by about 70 percent. Under these conditions $10^{-5}M$ DA failed to stimulate adenylate cyclase in homogenates of the SN from the lesioned side, whereas adenylate cyclase from the control side was stimulated 58 percent by DA (Table 1).

These results demonstrate that DA-sensitive adenylate cyclase in the SN is dependent on the presence of an anatomically restricted afferent projection from the forebrain.

Our electrolytic lesions did not significantly damage nigrostriatal DA neurons (Table 1). Moreover it is unlikely that serotonin (*7, 13*) or acetylcholine (*6*) neurons were damaged by these lesions or by the hemitransections anterior to the SN. Thus, in the SN there may exist a specific relationship between DA-sensitive adenylate cyclase and neurons containing GABA or substance P. The possibility that DA, released from dendrites in the SN, interacts with DA receptors located on terminals containing GABA or substance P is supported by evidence that these terminals make close synaptic contact with dendrites of DA neurons in the SN (*4, 16*). Since GABA and substance P respectively influence firing rates of nigral DA neurons (*17, 18*), there may exist a mutual interregulation between these systems. The respective contributions of GABA- and substance P–containing terminals to the DA-sensitive adenylate cyclase content of the SN remain to be determined.

It has been reported that iontophoretic application of DA (but not of amphetamine) causes a reduction in the activity of DA neurons due to an activation of autoreceptors (*19*). The reported inhibitory action of locally applied DA in the SN may not be mediated by autoreceptors but instead may be dependent on interactions with non-DA neurons which contain DA receptors. For example, if DA interacts with receptors on GABA terminals, the consequent release of GABA could account for the observed inhibitory actions of DA.

Studies of striatal DA-sensitive adenylate cyclase in other laboratories have provided evidence that this enzyme is not located on terminals of DA neurons. Destruction of striatal DA neurons with 6-OHDA did not reduce the degree of DA stimulation of cyclic AMP synthesis (*20, 21*). Together with our results in the SN, these findings cast doubt on the existence of DA autoreceptors. If such au-

Table 1. Dopamine-sensitive adenylate cyclase in the substantia nigra: effect of discrete unilateral electrocoagulation of striatonigral projections. Electrolytic lesions were made stereotaxically by passing cathodal current (1.0 ma for 10 seconds) from a constant direct-current lesion generator through a stainless steel electrode insulated except for 1 mm at the tip. Electrode placement was anteroposterior 3.8 mm, lateral 3.0 mm, and dorsoventral −2.5 mm (*28*). The lesion diameter was approximately 0.6 mm. Seven days after operation the animals were killed by decapitation. Nigral tissue from each animal was homogenized and divided into portions for assay of adenylate cyclase, GABA, and substance P, respectively. Adenylate cyclase, TH, and proteins were measured as described in the legend of Fig. 1; GABA (nanomoles per milligram of protein) was determined by the method of Okada *et al.* (*29*). Substance P was measured with radioimmunoassay method (*15*). Each value represents the mean ± standard deviation of values obtained from four animals.

Side	Nigral adenylate cyclase: cyclic AMP formed			Nigral GABA (nmole mg^{-1})	Nigral substance P (pmole mg^{-1})	Striatal TH (nmole mg^{-1})
	− DA (pmole mg^{-1} min^{-1})	$+10^{-5}M$ DA (pmole mg^{-1} min^{-1})	Increase after DA (%)			
Intact	38 ± 4.7	60 ± 6.3*	58	82 ± 5	13.8 ± 1.2	6.1 ± 0.92
Lesioned	39 ± 4.1	41 ± 4.0	5	23 ± 2*	3.9 ± 0.6*	5.3 ± 0.85

*$P < .05$ compared with the respective controls.

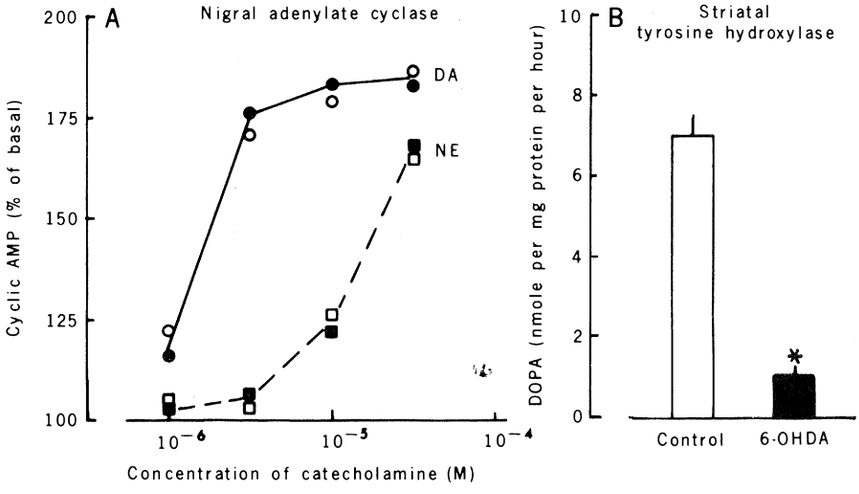

Fig. 1. Nigral adenylate cyclase (A) and striatal tyrosine hydroxylase (B) of control and 6-OHDA–treated hemispheres. By use of a stereotaxic technique, 6-OHDA (8 μg per 4 μl per 10 minutes) was infused directly into one substantia nigra (*22*) of Sprague-Dawley male rats (250 to 300 g) 10 to 15 days before they were killed. Animals were killed by decapitation and the frozen brains were serially sectioned (400-μm coronal sections; knife angle 30° to brain axis) in a cryostat (−5°C). Substantia nigra (zona compacta and zona reticulata) was carefully microdissected out from the surrounding white matter in sections corresponding to A1610 to A2580 (*23*). The striatum (caudate putamen) was similarly dissected out from sections corresponding to A6360 to A8920. The amount of proteins obtained from a single SN was between 200 and 300 μg; from striatum, 1.0 to 2.0 mg. (A) For the measurement of adenylate cyclase activity, the tissue was homogenized and incubated according to procedures described by Kebabian *et al.* (*24*); 25.0 μg of protein was used as the enzyme source. After purification on alumina and Dowex columns, the cyclic AMP formed was determined by measuring the amount of histone phosphorylation catalyzed by a cyclic AMP–dependent protein kinase (*25*). Basal adenylate cyclase activity was 32 ± 1.8 pmole mg^{-1} min^{-1} in 6-OHDA–treated SN ($N=5$). The increase of cyclic AMP obtained after DA or NE stimulation is expressed as percentage of basal activity. The concentrations of DA and NE on the abscissa are given on a logarithmic scale. Each point represents the mean for five separate experiments. The standard deviation of each value was 10 to 15 percent of the mean. Open symbols represent the 6-OHDA–treated side, closed symbols the control side. (B) For TH assay, the procedure was as described by Zivkovic *et al.* (*26*), except that 0.1 mM tyrosine and 1.5 mM 6,7-dimethyl-5,6,7,8-tetrahydropterine were used. Proteins were determined by the method of Lowry *et al.* (*27*). Each value represents the mean ± standard error of five determinations. (*) $P < .01$ compared with controls.

toreceptors do in fact exist, then they must be functionally unrelated to DA-dependent adenylate cyclase.

K. GALE
A. GUIDOTTI
E. COSTA

Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeths Hospital, Washington, D.C. 20032

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12. Cerebral hemitransection was done by blunt dissection, using a stainless steel No. 2 insect pin mounted in a Kopf electrode carrier. Sprague-Dawley male rats (300 g) were placed in a stereotaxic holder (tooth bar 5 mm above interaural line). The electrode carrier was inclined on the anterior-posterior plane at a 6.3° angle from vertical so that the lower tip of the insect pin was directed rostrally. The pin entered the cortex at anteroposterior 3.0 mm (*28*), was lowered 9.0 mm from the dura (just caudal to the premammillary nucleus), and was moved 5 mm (back and forth three to four times) laterally from the midline. The effectiveness of the transection was ascertained by measurement of TH activity in the forebrain. The lesion decreased forebrain TH by at least 85 percent. Each value represents the mean ± standard error for five animals.
13. Hemitransection posterior to the SN was performed as described in (*12*) except that the insect pin was inserted vertically just caudal to the SN. In these animals no change in TH was found in the ipsilateral striatum. The completeness of these lesions was verified histologically. Serotonergic pathways traveling rostrally from the midbrain raphe would be eliminated by this transection (*7*).
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Ornithine Decarboxylase May Function as an Initiation Factor for RNA Polymerase I

Abstract. *Reports suggest that the activity of RNA polymerase I is modulated by a labile protein with a half-life of 10 to 20 minutes. Ornithine decarboxylase is the only labile protein (half-life, 10 to 20 minutes) that increases in activity prior to increased RNA polymerase I activity. The addition of a small amount of a highly purified ornithine decarboxylase preparation to an RNA polymerase I assay increases the initial rate of the reaction as well as the time for which the assay is linear. The incorporation patterns of ^{14}C-labeled adenosine triphosphate and ^{32}P-labeled adenosine triphosphate into RNA indicate that the addition of ornithine decarboxylase to the RNA polymerase assay increases the rate of initiation. This report demonstrates a novel way to purify ornithine decarboxylase by RNA polymerase I affinity chromatography and presents data in support of the hypothesis that the labile protein which modulates RNA polymerase I activity is ornithine decarboxylase.*

Reports indicate that the control of RNA polymerase I (E.C. 2.7.7.6) activity is not through increased synthesis, de novo, of the enzyme, but rather through a modification of the enzyme structure that facilitates the attachment of this enzyme at the ribosomal DNA gene sites (*1*). The activity of RNA polymerase I appears to be dependent upon the presence of an extremely labile protein that is sensitive to amino acid pool sizes (*2*). Ornithine decarboxylase (ODC; E.C. 4.1.1.17), the first enzyme of the polyamine biosynthetic pathway, has the properties of such a protein (*3*). Additional evidence for an interrelationship between these two proteins includes (i) the early, rapid increase in ODC activity which immediately precedes increased RNA polymerase I activity after stimulation with any of a wide variety of trophic hormones (*4, 5*); (ii) inhibitor studies which indicate that any attenuation of ODC synthesis is reflected in a similar attenuation of RNA polymerase I activity (*5*); and (iii) ODC activity declines with a half-life of 15 minutes after treatment with cycloheximide; in this system, the activity of RNA polymerase I shows a lag period of 15 minutes and thereafter declines in activity with a slope of decline identical to that of ODC (*5, 6*).

Table 1. Purification of ODC from rat liver. The rats were injected with 3-isobutyl, 1-methylxanthine (10 mg/kg, in 0.9 percent saline with ethanol, 20 percent by volume) 4 hours prior to being killed.

Fraction	Volume (ml)	Protein (mg)	Total activity	Purification	Recovery (%)
Supernatant	186	4022.3	210.4		
Acetic acid precipitate	58	248.9	137.6	11	65
DEAE-cellulose	15.3	74.0	81.5	22	39
Affinity chromatography	2.0	0.8	79.2	1980	38